

Juha Punnonen, et al.  
Application No.: 09/247,886  
Page 2

**AMENDMENTS TO THE CLAIMS:**

Pursuant to the proposed revisions to 37 C.F.R. § 1.121, please amend the claims as follows. The following listing of claims replaces all prior versions and listings of claims in the application:

**Listing of Claims:**

1. (Canceled)
2. (Currently Amended) A method for obtaining a recombinant cell-specific binding moiety for an ability to increase uptake or specificity of a genetic vaccine for a target cell, the method comprising:
  - (1) recombining at least first and second forms of at least one nucleic acid, wherein each of the first and second forms of the nucleic acid comprises a polynucleotide that encodes a nucleic acid binding domain and at least first and second forms of at least one additional nucleic acid, wherein each of the first and second forms of the additional nucleic acid comprises a polynucleotide that encodes a cell-specific ligand that specifically binds to a protein on the surface of a cell of interest, wherein the first and second forms of each nucleic acid differ from each other in two or more nucleotides, to produce a library of recombinant binding moiety-encoding nucleic acids, wherein a recombinant binding moiety-encoding nucleic acid comprises a polynucleotide encoding a recombinant nucleic acid binding domain fused or covalently linked to a polynucleotide encoding a recombinant cell-specific ligand;
  - (2) producing a library of vectors from the library of recombinant binding moiety-encoding nucleic acids, wherein each vector comprises: a) a binding site specific for the nucleic acid binding domain and b) a member of the library of recombinant binding moiety-encoding nucleic acids;
  - (3) introducing one or more members of the library of vectors into one or more host cells, wherein the encoded recombinant binding moiety is expressed and recovering the expressed recombinant binding moiety;
  - (4) binding the expressed recombinant binding moiety to a vector comprising the binding site to form a vector-binding moiety complex;

Juha Punnonen, et al.  
Application No.: 09/247,886  
Page 3

(5) contacting the vector-binding moiety complex with ~~a~~ one or more target ~~cells~~ cell of interest; ~~and~~

(6) determining if one or more target cells contain a vector from the vector-binding moiety complex, comparing the percentage of target cells containing the vector from the vector-binding moiety complex with the percentage of target cells containing a control binding moiety after contacting the control binding moiety with one or more target cells, wherein the control binding moiety comprises a nucleic acid binding domain and cell-specific ligand of (1), and recovering the recombinant cell-specific binding moiety nucleic acid from any such target cells.

3. (Currently Amended) The method of claim 2, wherein the method further comprises:

(7) recombining at least one recombinant binding moiety-encoding nucleic acid of (6) with a further form of the polynucleotide that encodes a nucleic acid binding domain and/or a further form of the polynucleotide that encodes a cell-specific ligand, which are the same or different from the first and second forms, to produce a further library of recombinant binding moiety-encoding nucleic acids;

(8) producing a further library of vectors from the further library of recombinant binding moiety-encoding nucleic acids, wherein each vector in the further library of vectors comprises: a) a binding site specific for the nucleic acid binding domain and b) a member of the further library of recombinant binding moiety-encoding nucleic acids;

(9) introducing one or more members of the further library of vectors into one or more host cells, wherein the encoded recombinant binding moiety is expressed and recovering the expressed recombinant binding moiety;

(10) binding the expressed recombinant binding moiety of (9) to a vector comprising the binding site to form a vector-binding moiety complex;

(11) contacting the vector-binding moiety complex of (10) with a target cell of interest, ~~and~~ determining if one or more target cells contain a vector from the vector-binding moiety complex of (10) and comparing the percentage of target cells containing the

HI  
Cont

Juha Punnonen, et al.  
Application No.: 09/247,886  
Page 4

vector from the vector-binding moiety complex with the percentage of target cells containing the control binding moiety after contacting the control binding moiety with one or more target cells;

(12) recovering the recombinant binding moiety-encoding nucleic acid from any such target cells of (11); and

(13) repeating (7) through (12) to screen for a cell-specific binding moiety useful for increasing uptake or specificity of a genetic vaccine vector for a target cell.

4. (Previously Presented) The method of claim 2, wherein the method comprises screening for one or more cell-specific binding moieties that increase uptake of a genetic vaccine vector by the target cells.

5. (Previously Presented) The method of claim 2, wherein the nucleic acid binding domain is a DNA binding domain derived from a protein selected from the group consisting of a transcriptional regulator, a polypeptide involved in DNA replication or recombination, a repressor, a histone, a protamine, an E. coli CAP protein, myc, a protein having a leucine zipper, a protein having a DNA binding basic domain, a protein having a POU domain, a protein having a zinc finger, and a protein having a Cys3His (SEQ ID NO:6) box.

6. (Original) The method of claim 2, wherein the nucleic acid binding domain is an RNA binding domain derived from a protein selected from the group consisting of HIV *tat* and HIV *rev*.

7. (Previously Presented) The method of claim 2, wherein the target cell of interest is selected from the group consisting of muscle cells, monocytes, dendritic cells, B cells, T cells, Langerhans cells, keratinocytes, M-cells, liver cells and epithelial cells.

8. (Previously Presented) The method of claim 7, wherein the target cell of interest is a professional antigen presenting cell.

9. (Original) The method of claim 8, wherein the antigen presenting cell is a dendritic cell, a monocyte/macrophage, a B cell, or a Langerhans cell.

10. (Previously Presented) The method of claim 8, wherein the cell-specific ligand comprises a polypeptide derived from a protein selected from the group consisting of CD2, CD28, CTLA-4, CD40 ligand, fibrinogen, ICAM-1, Fc portion of immunoglobulin G, and a bacterial enterotoxin, or a subunit thereof.

Juha Punnonen, et al.  
Application No.: 09/247,886  
Page 5

11. (Original) The method of claim 2, wherein the target cell of interest is a human cell.

12. (Previously Presented) The method of claim 2, wherein target cells that contain the vector are identified by selecting for expression of a selectable marker contained in the vector.

13. (Previously Presented) The method of claim 2, wherein each recombinant binding moiety-encoding nucleic acid comprises a genetic vaccine vector.

14-16. (Canceled)

17. (Previously Presented) A composition for eliciting an immune response that comprises:

a) a recombinant binding moiety that comprises a nucleic acid binding domain and a cell-specific ligand, and

b) a polynucleotide sequence that is capable of expressing an antigen and that comprises a binding site, wherein the nucleic acid binding domain is capable of specifically binding to the binding site.

18. (Currently Amended) A method for obtaining a recombinant cell-specific binding moiety for an ability to increase uptake, efficacy, or specificity of a vaccine or antigen for a target cell, the method comprising:

(1) recombining at least first and second forms of at least one nucleic acid, wherein each of the first and second forms of the nucleic acid comprises a polynucleotide which encodes a binding moiety polypeptide of an enterotoxin, wherein the first and second forms differ from each other in two or more nucleotides, to produce a library of recombinant nucleic acids;

(2) producing a library of vectors from the library of recombinant nucleic acids, wherein each vector comprises a member of the library of recombinant nucleic acids;

(3) introducing one or more members of the library of vectors into one or more host cells, wherein the one or more members of the library of recombinant nucleic acids are expressed to form one or more recombinant cell-specific binding moiety polypeptides and recovering the one or more recombinant cell-specific binding moiety polypeptides;

Juha Punnonen, et al.  
Application No.: 09/247,886  
Page 6

(4) contacting the one or more recombinant cell-specific binding moiety polypeptides with a cell surface receptor of a target cell; and

(5) determining which of the one or more recombinant cell-specific binding moiety polypeptides exhibits an enhanced ability to bind to the target cell compared to the ability of a binding moiety polypeptide ~~needed~~ of (1) to bind to the target cell.

19. (Previously Presented) The method of claim 18, wherein the cell surface receptor is present on the surface of a target cell during said contacting.

20. (Original) The method of claim 18, wherein the cell surface receptor is  $G_{M1}$ .

21. (Original) The method of claim 18, wherein the host cell is a *V. cholerae* cell which is incapable of expressing CT-A.

22. (Previously Presented) A method for producing a composition for eliciting an immune response, the method comprising coating a polynucleotide that is capable of expressing an antigen with a recombinant cell-specific binding moiety polypeptide produced by the method of claim 18.

23. (Previously Presented) The method of claim 18, wherein each of the one or more recombinant cell-specific binding moiety polypeptides is expressed as a fusion protein on the surface of a replicable genetic package.

24-50. (Canceled)

51. (Previously Presented) A method for obtaining a recombinant cell-specific binding moiety polypeptide for an ability to increase uptake, efficacy, or specificity of a vaccine antigen for a target cell, the method comprising:

(1) recombining at least first and second forms of at least one nucleic acid that comprises a polynucleotide which encodes a cell-specific binding moiety polypeptide, wherein the first and second forms differ from each other in two or more nucleotides, to produce a library of recombinant nucleic acids;

(2) introducing one or more members of a library of vectors, each of which comprises a member of the library of recombinant nucleic acids, into one or more host

Juha Punnonen, et al.  
Application No.: 09/247,886  
Page 7

cells, wherein one or more members of the library of recombinant nucleic acids are expressed to form one or more recombinant cell-specific binding moiety polypeptides;

(3) contacting the one or more recombinant cell-specific binding moiety polypeptides with a cell surface receptor of a target cell;

(4) determining which of the one or more recombinant cell-specific binding moiety polypeptides exhibits an enhanced ability to bind to the target cell compared to the ability of the cell-specific binding moiety polypeptide of (1) to bind the target cell; and

(5) fusing or linking the recombinant cell-specific binding moiety polypeptide to the vaccine antigen or coating the vaccine antigen with the recombinant cell-specific binding moiety polypeptide.

52. (Previously Presented) The method of claim 51, wherein each of the one or more recombinant cell-specific binding moiety polypeptides is expressed as a fusion protein on the surface of a replicable genetic package.

53. (Previously Presented) The method of claim 51, wherein each of the one or more recombinant cell-specific binding moiety polypeptides is fused or linked to the vaccine antigen.

54. (Previously Presented) The method of claim 51, wherein the target cell is selected from the group consisting of muscle cells, monocytes, dendritic cells, B cells, T cells, Langerhans cells, keratinocytes, M-cells, liver cells and epithelial cells.

55. (Previously Presented) The method of claim 51, wherein the cell surface receptor is present on the surface of a target cell during said contacting.

56. (Previously Presented) The method of claim 51, wherein each of the cell-specific binding moiety polypeptides comprises a polypeptide derived from a protein selected from the group consisting of selected from the group consisting of CD2, CD28, CTLA-4, CD40, and ligands thereof; fibrinogen; factor X; ICAM-1;  $\beta$ -glycan; Fc portion of immunoglobulin G; and a bacterial enterotoxin, or a subunit thereof.

57. (Previously Presented) A method for producing a composition for eliciting an immune response, said method comprising coating an antigen with a recombinant cell-specific binding moiety polypeptide produced by the method of claim 51.

Juha Punnonen, et al.  
Application No.: 09/247,886  
Page 8

58. (Previously Presented) A composition for eliciting an immune response comprising an antigen and a recombinant cell-specific binding moiety polypeptide, wherein the composition is produced by the method of claim 51, wherein:

- (i) the antigen comprises a polypeptide antigen;
- (ii) the cell-specific binding moiety polypeptide exhibits enhanced ability to bind to the target cell; and
- (iii) the polypeptide antigen and the recombinant cell-specific binding moiety polypeptide are derived from different polypeptides.

59. (Previously Presented) The method of claim 2, wherein the binding site of each vector is derived from a binding site present in at least one form of at least one nucleic acid of (1).

60. (Previously Presented) The method of claim 2, wherein the binding site is joined to the member of the library of recombinant binding moiety-encoding nucleic acids after said recombining.

61. (Previously Presented) The method of claim 2, wherein the vector-binding moiety complex forms inside the host cell and, prior to the contacting of (5), the host cell is lysed under conditions that do not disrupt the vector-binding moiety complex.

62. (Previously Presented) The method of claim 3, wherein the vector-binding moiety complex of (10) forms inside the host cell and, prior to the contacting of (11), the host cell is lysed under conditions that do not disrupt the vector-binding moiety complex.

63. (Previously Presented) The method of claim 2, wherein the cell-specific ligand comprises a polypeptide derived from a protein selected from the group consisting of CD2, CD28, CTLA-4, CD40, and ligands therefor; fibrinogen; factor X; ICAM-1;  $\beta$ -glycan; Fc portion of immunoglobulin G; and a bacterial enterotoxin, or a subunit thereof.

64. (Previously Presented) The method of claim 51, wherein the vaccine antigen is coated with one of the one or more recombinant cell-specific binding moiety polypeptides.